

Rec'd PCT/PTO 21 JAN 2005 PATENT COOPERATION TREATY
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference REG/G18698WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/GB 03/02919	International filing date (day/month/year) 04.07.2003	Priority date (day/month/year) 22.07.2002
International Patent Classification (IPC) or both national classification and IPC A61K51/04		
Applicant PSIMEI PHARMACEUTICALS PLC et al.		



1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 23.02.2004	Date of completion of this report 05.11.2004
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Döpfer, K-P Telephone No. +49 89 2399-8547 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**International application No. **PCT/GB 03/02919****I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-25 as originally filed

Claims, Numbers

1-15 filed with telefax on 07.07.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☒ the claims, Nos.: 16-18
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**International application No. **PCT/GB 03/02919****V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement****1. Statement**

Novelty (N)	Yes: Claims	1-15
	No: Claims	
Inventive step (IS)	Yes: Claims	1-15
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations**see separate sheet**

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/02919

Re Item I**Basis of the report**

1. The amended claims 1-15 comply with the requirements of Article 34(2) PCT.

Re Item V**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Novelty (Article 33(2) PCT)

- 1.1 Reference is made to the following documents:

- D1: J. Control. Release 74(1-3):147-158(2001)
- D2: US-A-6 017 902,
- D3: J. Control. Release 75(3):365-379(2001),
- D4: Int. J. Cancer 86(1):108-117(2000),
- D5: Ann. NY Acad. Sci. 618:335-344(1991)
- D6: J. Control. Release 74(1-3):249-253(2001),
- D7: Nucl. Med. Biol. 29(2):159-167(2002),
- D8: J. Control. Release 54(2):223-233(1998),
- D9: J. Control. Release 61(1-2):145-157(1999),
- D10: J. Control. Release 78(1-3):165-173(2002),
- D11: J. Control. Release 74(1-3):203-211(2001)

- 1.2 The present application addresses conjugates for e.g. neutron capture therapy having the general formula $P-(L-NAT)_n$ (I) with P representing a hydroxypropylmethacrylamide-methacrylate copolymer (Poly(HPMA-co-MA)); NAT representing a nuclide activation therapy reagent, and L representing a linker moiety, n represents an integer from 1 to 1,000. The conjugate must further comprise a chemotherapeutic agent attached to the polymer via the linker L. Further particular subject-matter claimed comprise conjugates of (Poly(HPMA-co-MA) with the linker molecules Gly-Gly, and Gly-Phe-Leu-Gly respectively and the anti-cancer compounds boronocaptate melphalan (BSMel) further linked with Paclitaxel (Pac) or Doxorubicin (Dox).

- 1.3 None of the prior art documents D1 to D11 disclose neither conjugates with the general formula (I) nor the particular entities with BSMel and/or Pax/Dox. Thus,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/02919

present claims 1-15 are novel.

2. Inventive Step (Article 33(3) PCT)

- 2.1 Any of the documents D1, D3-D6 and D8-D11 discloses (Poly(HPMA-co-MA) conjugated to anti-cancer compounds. These conjugates are used for targeted delivery of the conjugated drugs to tumour tissue. Documents D5 and D6 discuss a combination of chemotherapy and photodynamic therapy with (Poly(HPMA-co-MA) conjugates comprising Dox/mesochlorine e_6 . Concerning the polymeric part, any of the prior art documents D1, D3-D6 or D8-D11 can be considered as representing the closest prior art. D5 and D6 are particularly relevant due to their additional focus on photodynamic therapy which could be considered as being similar to the neutron capture therapy.

The problem underlying the present application is to be regarded as to provide further conjugates for the targeted delivery of nuclide activation therapy agents (NATs) to tumour cells.

The solution are conjugates of the general formula (I) varying in the composition concerning (Poly(HPMA-co-MA), L, and NAT, i.e a delivery vehicle which allows the combination of chemotherapy and radiotherapy. Although all elements are disclosed in the prior art, no qualified hint is given to provide a conjugate which combines elements of chemotherapy with those of radiotherapy. Taking the knowledge of photodynamic therapy into consideration, the skilled person has no realistic expectation of success when adapting and combining this concept with that of a normal conjugate of a chemotherapeutic with a delivery vehicle. The advantage of this administration regimen is that the patient can be provided in one session with two differently acting anti-cancer agents, which increases the efficacy of the therapy of tumours which are susceptible to chemotherapeutic agents as well as to radiotherapy.

The subject-matter presently claimed is thus not directly derivable from the teaching of the prior art and appears not to be obvious. Inventive step can be acknowledged for the subject-matter of present claims 1-15.

3. Industrial applicability (Article 33(4) PCT)

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EXAMINATION REPORT - SEPARATE SHEET

The subject-matter of present claims 1-15 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.

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Claims

1. A conjugate having the general formula

5 P-(L-NAT)_n

wherein

10 P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a molecular weight of 5-6,000 kDa;

NAT represents a nuclide activation therapy agent;

L represents a linker moiety capable of linking the polymer to the neutron capture therapy agent; and

15 n represents an integer from 1-1,000

and wherein the conjugate further comprises a chemotherapeutic agent attached to the polymer via the linker moiety L.

20 2. A conjugate as claimed in claim 1, wherein the polymer is a 2-hydroxypropylmethacrylamide-methacrylate copolymer.

3. A conjugate as claimed in any preceding claim, wherein the polymer has a molecular weight of 5-100, preferably 10-70, more preferably 15-45, most preferably 20-40 kDa.

25 4. A conjugate as claimed in any preceding claim, wherein the ratio of hydroxypropylmethacrylamide to methacrylate is from 20:1 to 1:1.

5. A conjugate as claimed in any preceding claim, wherein the nuclide activation therapy agent is a neutron capture therapy agent.

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E.P.O MUNICH

07-07-2004

6. A conjugate as claimed in claim 5, wherein the neutron capture therapy agent contains at least one nuclide selected from ^6Li , ^{10}B , ^{22}Na , ^{58}Co , ^{113}Cd , ^{126}I , ^{135}Xe , ^{148}Pm , ^{149}Sm , ^{151}Eu , ^{153}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U and ^{241}Pu in sufficient quantity to undergo a neutron capture reaction.

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7. A conjugate as claimed in claim 6, wherein the nuclide is ^{10}B .

8. A conjugate as claimed in any of claims 5 to 7, wherein NAT represents a boronated amino acid or peptide, a modified carborane cage, a mercaptoborate, a boron-containing porphyrin or phthalocyanine, a boron-containing nucleic acid precursor, or a boron-containing foliate growth factor, hormone, radiation sensitiser, phosphates, phosphonate, phosphoramidates, cyclic thiourea derivative, amine, promazine, hydantoin or barbiturate.

9. A conjugate as claimed in any preceding claim, wherein the NAT agent makes up 1-30%, preferably 5-10%, of the overall mass of the conjugate.

10. A conjugate as claimed in any preceding claim, wherein the linker represents a linear or branched C_{1-15} alkyl which may be saturated or unsaturated, optionally substituted by carbonyl, amide, hydroxyl or halogen; a peptide, preferably 1-10 amino acids in length, in which the amino acids may be further substituted with amino, thio, carboxyl, carboxamide or imidazole groups; or a covalent bond.

11. A conjugate as claimed in any preceding claim, wherein n represents an integer from 1-500, preferably 1-100, particularly preferably 1-20.

25

12. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMel) Gly-Phe-Leu-Gly-Paclitaxel.

13. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMel) Gly-Phe-Leu-Gly-Doxorubicin.

14. A pharmaceutical composition containing the conjugate as claimed in any preceding claim.

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ELKINGTON & FIFE LONDON → E.P.O MUNICH

07/07/2004

15. Use of the conjugate as claimed in any of claims 1-13 for the preparation of a medicament for the treatment of cancer.

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)

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International application No. PCT/GB 03/02919	International filing date (day/month/year) 04.07.2003	Priority date (day/month/year) 22.07.2002
International Patent Classification (IPC) or both national classification and IPC A61K51/04		
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- II ☐ Priority
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- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 23.02.2004	Date of completion of this report 05.11.2004
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Döpfer, K-P Telephone No. +49 89 2399-8547 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/GB 03/02919**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-25 as originally filed

Claims, Numbers

1-15 filed with telefax on 07.07.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
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3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☒ the claims, Nos.: 16-18
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/GB 03/02919**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-15
	No: Claims	
Inventive step (IS)	Yes: Claims	1-15
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/02919

Re Item I

Basis of the report

1. The amended claims 1-15 comply with the requirements of Article 34(2) PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty (Article 33(2) PCT)

- 1.1 Reference is made to the following documents:

D1: J. Control. Release **74**(1-3):147-158(2001)
D2: US-A-6 017 902,
D3: J. Control. Release **75**(3):365-379(2001),
D4: Int. J. Cancer **86**(1):108-117(2000),
D5: Ann. NY Acad. Sci. **618**:335-344(1991)
D6: J. Control. Release **74**(1-3):249-253(2001),
D7: Nucl. Med. Biol. **29**(2):159-167(2002),
D8: J. Control. Release **54**(2):223-233(1998),
D9: J. Control. Release **61**(1-2):145-157(1999),
D10: J. Control. Release **78**(1-3):165-173(2002),
D11: J. Control. Release **74**(1-3):203-211(2001)

- 1.2 The present application addresses conjugates for e.g. neutron capture therapy having the general formula $P-(L-NAT)_n$ (I) with P representing a hydroxypropylmethacrylamide-methacrylate copolymer (Poly(HPMA-co-MA)); NAT representing a nuclide activation therapy reagent, and L representing a linker moiety, n represents an integer from 1 to 1,000. The conjugate must further comprise a chemotherapeutic agent attached to the polymer via the linker L. Further particular subject-matter claimed comprise conjugates of (Poly(HPMA-co-MA) with the linker molecules Gly-Gly, and Gly-Phe-Leu-Gly respectively and the anti-cancer compounds boronocaptate melphalan (BSMel) further linked with Paclitaxel (Pac) or Doxorubicin (Dox).

- 1.3 None of the prior art documents D1 to D11 disclose neither conjugates with the general formula (I) nor the particular entities with BSMel and/or Pax/Dox. Thus,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/02919

present claims 1-15 are novel.

2. Inventive Step (Article 33(3) PCT)

- 2.1 Any of the documents D1, D3-D6 and D8-D11 discloses (Poly(HPMA-co-MA) conjugated to anti-cancer compounds. These conjugates are used for targeted delivery of the conjugated drugs to tumour tissue. Documents D5 and D6 discuss a combination of chemotherapy and photodynamic therapy with (Poly(HPMA-co-MA) conjugates comprising Dox/mesochlorine e_6 . Concerning the polymeric part, any of the prior art documents D1, D3-D6 or D8-D11 can be considered as representing the closest prior art. D5 and D6 are particularly relevant due to their additional focus on photodynamic therapy which could be considered as being similar to the neutron capture therapy.

The problem underlying the present application is to be regarded as to provide further conjugates for the targeted delivery of nuclide activation therapy agents (NATs) to tumour cells.

The solution are conjugates of the general formula (I) varying in the composition concerning (Poly(HPMA-co-MA), L, and NAT, i.e a delivery vehicle which allows the combination of chemotherapy and radiotherapy. Although all elements are disclosed in the prior art, no qualified hint is given to provide a conjugate which combines elements of chemotherapy with those of radiotherapy. Taking the knowledge of photodynamic therapy into consideration, the skilled person has no realistic expectation of success when adapting and combining this concept with that of a normal conjugate of a chemotherapeutic with a delivery vehicle. The advantage of this administration regimen is that the patient can be provided in one session with two differently acting anti-cancer agents, which increases the efficacy of the therapy of tumours which are susceptible to chemotherapeutic agents as well as to radiotherapy.

The subject-matter presently claimed is thus not directly derivable from the teaching of the prior art and appears not to be obvious. Inventive step can be acknowledged for the subject-matter of present claims 1-15.

3. Industrial applicability (Article 33(4) PCT)

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/02919

The subject-matter of present claims 1-15 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.

Claims

1. A conjugate having the general formula

5 P-(L-NAT)_n

wherein

P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a
10 molecular weight of 5-6,000 kDa;

NAT represents a nuclide activation therapy agent;

L represents a linker moiety capable of linking the polymer to the neutron capture therapy
agent; and

n represents an integer from 1-1,000.

15 2. A conjugate as claimed in claim 1, wherein the polymer is a 2-hydroxypropylmethacrylamide-methacrylate copolymer.

3. A conjugate as claimed in any preceding claim, wherein the polymer has a molecular
20 weight of 5-100, preferably 10-70, more preferably 15-45, most preferably 20-40 kDa.

4. A conjugate as claimed in any preceding claim, wherein the ratio of
hydroxypropylmethacrylamide to methacrylate is from 20:1 to 1:1.

25 5. A conjugate as claimed in any preceding claim, wherein the nuclide activation
therapy agent is a neutron capture therapy agent.

6. A conjugate as claimed in claim 5, wherein the neutron capture therapy agent
contains at least one nuclide selected from ⁶Li, ¹⁰B, ²²Na, ⁵⁸Co, ¹¹³Cd, ¹²⁶I, ¹³⁵Xe, ^{148m}Pm,
30 ¹⁴⁹Sm, ¹⁵¹Eu, ¹⁵⁵Gd, ¹⁵⁷Gd, ¹⁶⁴Dy, ¹⁸⁴Os, ¹⁹⁹Hg, ²³⁰Pa, ²³⁵U and ²⁴¹Pu in sufficient quantity
to undergo a neutron capture reaction.

REPLACED BY
ART 34 AMDT

7. A conjugate as claimed in claim 6, wherein the nuclide is ^{10}B .
8. A conjugate as claimed in any of claims 5 to 7, wherein NAT represents a boronated amino acid or peptide, a modified carborane cage, a mercaptoborate, a boron-containing porphyrin or phthalocyanine, a boron-containing nucleic acid precursor, or a boron-containing foliate growth factor, hormone, radiation sensitiser, phosphates, phosphonate, phosphoramidates, cyclic thiourea derivative, amine, promazine, hydantoin or barbiturate.
9. A conjugate as claimed in any preceding claim, wherein the NAT agent makes up 1-30%, preferably 5-10%, of the overall mass of the conjugate.
10. A conjugate as claimed in any preceding claim, wherein the linker represents a linear or branched C_{1-15} alkyl which may be saturated or unsaturated, optionally substituted by carbonyl, amide, hydroxyl or halogen; a peptide, preferably 1-10 amino acids in length, in which the amino acids may be further substituted with amino, thio, carboxyl, carboxamide or imidazole groups; or a covalent bond.
11. A conjugate as claimed in any preceding claim, wherein n represents an integer from 1-500, preferably 1-100, particularly preferably 1-20.
12. A conjugate as claimed in any preceding claim, further comprising a chemotherapeutic agent attached to the polymer via the linker moiety L .
13. Poly(HPMA-co-MA-GG-BSMeI).
14. Poly(HPMA-co-MA-GFLG-BSMeI).
15. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMeI) Gly-Phe-Leu-Gly-Paclitaxel.
16. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMeI) Gly-Phe-Leu-Gly-Doxorubicin.

REPLACED BY
ART 34 AMDT

17. A pharmaceutical composition containing the conjugate as claimed in any preceding claim.

- 5 18. Use of the conjugate as claimed in any of claims 1-16 for the preparation of a medicament for the treatment of cancer.

ART 34 AMDT

Claims

1. A conjugate having the general formula

5 P-(L-NAT)_n

wherein

P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a
10 molecular weight of 5-6,000 kDa;

NAT represents a nuclide activation therapy agent;

L represents a linker moiety capable of linking the polymer to the neutron capture therapy
agent; and

n represents an integer from 1-1,000

15

and wherein the conjugate further comprises a chemotherapeutic agent attached to the
polymer via the linker moiety L.

20

2. A conjugate as claimed in claim 1, wherein the polymer is a 2-
hydroxypropylmethacrylamide-methacrylate copolymer.

3. A conjugate as claimed in any preceding claim, wherein the polymer has a molecular
weight of 5-100, preferably 10-70, more preferably 15-45, most preferably 20-40 kDa.

25

4. A conjugate as claimed in any preceding claim, wherein the ratio of
hydroxypropylmethacrylamide to methacrylate is from 20:1 to 1:1.

5. A conjugate as claimed in any preceding claim, wherein the nuclide activation
therapy agent is a neutron capture therapy agent.

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ART 34 AMDT

6. A conjugate as claimed in claim 5, wherein the neutron capture therapy agent contains at least one nuclide selected from ^6Li , ^{10}B , ^{22}Na , ^{58}Co , ^{113}Cd , ^{126}I , ^{135}Xe , ^{148}Pm , ^{149}Sm , ^{151}Eu , ^{153}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U and ^{241}Pu in sufficient quantity to undergo a neutron capture reaction.

5

7. A conjugate as claimed in claim 6, wherein the nuclide is ^{10}B .

8. A conjugate as claimed in any of claims 5 to 7, wherein NAT represents a boronated amino acid or peptide, a modified carborane cage, a mercaptoborate, a boron-containing porphyrin or phthalocyanine, a boron-containing nucleic acid precursor, or a boron-containing foliate growth factor, hormone, radiation sensitiser, phosphates, phosphonate, phosphoramidates, cyclic thiourea derivative, amine, promazine, hydantoin or barbiturate.

9. A conjugate as claimed in any preceding claim, wherein the NAT agent makes up 1-30%, preferably 5-10%, of the overall mass of the conjugate.

10. A conjugate as claimed in any preceding claim, wherein the linker represents a linear or branched C_{1-15} alkyl which may be saturated or unsaturated, optionally substituted by carbonyl, amide, hydroxyl or halogen; a peptide, preferably 1-10 amino acids in length, in which the amino acids may be further substituted with amino, thio, carboxyl, carboxamide or imidazole groups; or a covalent bond.

11. A conjugate as claimed in any preceding claim, wherein n represents an integer from 1-500, preferably 1-100, particularly preferably 1-20.

25

12. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMeI) Gly-Phe-Leu-Gly-Paclitaxel.

13. Poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMeI) Gly-Phe-Leu-Gly-Doxorubicin.

14. A pharmaceutical composition containing the conjugate as claimed in any preceding claim.

ART 34 AWD

15. Use of the conjugate as claimed in any of claims 1-13 for the preparation of a medicament for the treatment of cancer.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



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(26) Publication Language: English

(30) Priority Data:
02255107.1 22 July 2002 (22.07.2002) EP

(71) Applicant (for all designated States except US): **PSIMEI PHARMACEUTICALS PLC** [GB/GB]; Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **PATEL, Bipin, C., M.** [GB/GB]; Psimei Pharmaceuticals PLC, Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).

(74) Agent: **ELKINGTON AND FIFE**; Prospect House, 8 Pembroke Road, Sevenoaks, Kent TN13 1XR (GB).

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(54) Title: NEW ANTI-CANCER COMPOUNDS

(57) Abstract: The present invention relates to new anti-cancer compounds and in particular to new compounds for use in nuclide activation therapy, such as neutron capture therapy. Specifically this invention provides a conjugate having the general formula P-(L-NAT)_n wherein P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a molecular weight of 5-6,000 kDa; NAT represents a nuclide activation therapy agent; L represents a linker moiety capable of linking the polymer to the neutron capture therapy agent; and n represents an integer from 1-1,000.



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- (71) Applicant (*for all designated States except US*): PSIMEI PHARMACEUTICALS PLC [GB/GB]; Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (*for US only*): PATEL, Bipin, C., M. [GB/GB]; Psimei Pharmaceuticals PLC, Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).
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(54) Title: CONJUGATES OF N-HYDROXYPROPYLMETHACRYLAMIDE-METHACRYLATE COPOLYMER WITH NUCLIDE ACTIVATION AGENT AND/OR ANTI-CANCER COMPOUNDS

(57) Abstract: The present invention relates to new anti-cancer compounds and in particular to new compounds for use in nuclide activation therapy, such as neutron capture therapy. Specifically this invention provides a conjugate having the general formula P-(L-NAT)_n wherein P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a molecular weight of 5-6,000 kDa; NAT represents a nuclide activation therapy agent; L represents a linker moiety capable of linking the polymer to the neutron capture therapy agent; and n represents an integer from 1-1,000.

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOPECEK J ET AL: "Water soluble polymers in tumor targeted delivery" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 74, no. 1-3, 6 July 2001 (2001-07-06), pages 147-158, XP004297520 ISSN: 0168-3659 the whole document	1-18
Y	US 6 017 902 A (CODERRE JEFFREY A ET AL) 25 January 2000 (2000-01-25) the whole document	1-18



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	<p>SAKUMA S ET AL: "Biorecognizable HPMA copolymer-drug conjugates for colon-specific delivery of 9-aminocamptothecin" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 75, no. 3, 10 August 2001 (2001-08-10), pages 365-379, XP004296352 ISSN: 0168-3659 the whole document</p>	1-18
Y	<p>MINKO T ET AL: "EFFICACY OF THE CHEMOTHERAPEUTIC ACTION OF HPMA COPOLYMER-BOUND DOXORUBICIN IN A SOLID TUMOR MODEL OF OVARIAN CARCINOMA" INTERNATIONAL JOURNAL OF CANCER, NEW YORK, NY, US, vol. 86, no. 1, 1 April 2000 (2000-04-01), pages 108-117, XP001027138 ISSN: 0020-7136 abstract page 109; figure 1 page 110, left-hand column, last paragraph -page 116, right-hand column, last paragraph</p>	1-18
Y	<p>KOPECEK J: "TARGETABLE POLYMERIC ANTICANCER DRUGS TEMPORAL CONTROL OF DRUG ACTIVITY" ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, NEW YORK ACADEMY OF SCIENCES, NEW YORK, NY, US, vol. 618, 1991, pages 335-344, XP001097388 ISSN: 0077-8923 page 337, last paragraph -page 342, line 2</p>	1-18
Y	<p>SHIAH J-G ET AL: "Combination chemotherapy and photodynamic therapy of targetable N-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin/mesochlorin e6-OV-TL 16 antibody immunoconjugates" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 74, no. 1-3, 6 July 2001 (2001-07-06), pages 249-253, XP004297531 ISSN: 0168-3659 the whole document</p>	1-18

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>NOVICK S ET AL: "Linkage of boronated polylysine to glycoside moieties of polyclonal antibody; boronated antibodies as potential delivery agents for neutron capture therapy"</p> <p>NUCLEAR MEDICINE AND BIOLOGY, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 29, no. 2, February 2002 (2002-02), pages 159-167, XP004334813</p> <p>ISSN: 0969-8051</p> <p>the whole document</p>	1-18
Y	<p>MINKO T ET AL: "HPMA copolymer bound adriamycin overcomes MDR1 gene encoded resistance in a human ovarian carcinoma cell line"</p> <p>JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 54, no. 2, 31 July 1998 (1998-07-31), pages 223-233, XP004134571</p> <p>ISSN: 0168-3659</p> <p>the whole document</p>	1-18
Y	<p>SHIAH J-G J-G ET AL: "Biodistribution of free and N-(2-hydroxypropyl)methacrylamide copolymer-bound mesochlorin e6 and adriamycin in nude mice bearing human ovarian carcinoma OVCAR-3 xenografts"</p> <p>JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 61, no. 1-2, 27 August 1999 (1999-08-27), pages 145-157, XP004362972</p> <p>ISSN: 0168-3659</p> <p>the whole document</p>	1-18
Y	<p>LU Z-R ET AL: "Design of novel bioconjugates for targeted drug delivery"</p> <p>JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 78, no. 1-3, 17 January 2002 (2002-01-17), pages 165-173, XP004329815</p> <p>ISSN: 0168-3659</p> <p>the whole document</p>	1-18

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KASUYA Y ET AL: "Synthesis and characterization of HPMACopolymer-aminopropylgeldanamycin conjugates"</p> <p>JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 74, no. 1-3, 6 July 2001 (2001-07-06), pages 203-211, XP004297525 ISSN: 0168-3659 the whole document</p> <p>-----</p>	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 03/02919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6017902	A	25-01-2000	NONE

New anti-cancer compounds

This invention relates to new anti-cancer compounds and in particular to new compounds for use in nuclide activation therapy, such as neutron capture therapy.

5

In nuclide activation therapy, a nuclide is activated and undergoes nuclear fission leading to the emission of highly ionizing radiation capable of destroying living cells. When the nuclide is activated using neutrons, the process is termed neutron capture therapy. The principle of neutron capture therapy (NCT) for the treatment for cancer was first
10 described in 1936 by the American scientist Locker. In essence, when an NCT element, for example the stable nuclide boron-10, is irradiated by non-ionizing slow neutrons, a fission reaction results that leads to the emission of highly ionizing radiation with a range of 7 to 9 μm . In 1951 the first patient was treated and since then the principle has been successfully demonstrated in the clinic although thus far effective NCT drugs have been
15 elusive.

Boron neutron capture therapy is a bimodal therapy requiring spatial and temporal overlap of neutrons and the drug. To achieve a biological effect, an interaction between slow neutrons and a boron carrying agent are necessary. The BNCT reaction of ^{10}B with
20 neutrons may be summarised by the following equation:



The 2.4 MeV energy is taken up as kinetic energy by the Li^+ and He^{2+} ions. The two
25 particles are sufficiently energetic to generate intense ionization tracks with a maximum range of 9 μm . Damage is thus confined to the diameter of a tumour cell, i.e. 10 μm . Accordingly, only the cells which contain ^{10}B are damaged, while non- ^{10}B containing (healthy) cells are left intact. The ^{10}B nuclide is stable and non-radioactive and its nucleus has a very large neutron absorption cross section for slow neutrons, i.e. 2 700
30 times greater than hydrogen. This translates into an ability to absorb neutrons of several

thousand times better than that of the elements constituting living tissues, such as hydrogen, oxygen and carbon.

BNCT can be used to treat cancers which are normally treated with radiotherapy, such as lymphomas and skin cancers, as well as cancers of the brain, breast, lung, head and neck, bone, prostate, pancreas and cervix. In addition, when surgical removal of a tumour is planned, BNCT may also be used to help reduce the size of the tumour and to reduce the associated normal tissue loss.

BNCT has been driven by the potential benefits of selective "in situ" radiotherapy and as a potential substitute to conventional X-ray radiotherapy. Over 500 patients have received experimental BNCT treatment for brain and skin tumours world wide. Predominantly, two experimental compounds 4-dihydroxyborylphenylalanine (BPA) and sodium mercaptoundecahydrododecaborate (BSH) have been used in the clinical trials.

In conventional radiotherapy the biological effect is spread over the entire irradiated area whilst with BNCT it is specific to those cells containing ^{10}B carrier molecules. A relatively high radiation dose is required with radiotherapy to generate the destructive ionization tracks for the biological effect. This is a limiting factor in the effectiveness of the treatment. Radiotherapy is limited by the inherent nature of the low LET (linear energy transfer) radiation beam which comprise of electrons (β -particles) or photons (X-rays and γ -rays). With BNCT short range high energy α and ^7Li particles are generated. These particles are high LET particles that are intensely ionizing and exhibit a greater more potent destructive propensity. Hence, the BNCT dose required to generate an equivalent radiotherapy biological effect is very much smaller than the relative radiotherapy dose.

Thus, in contrast to radiotherapy, BNCT offers target tissue selectivity via the specificity of the drug. Lethal radiation is only generated where ^{10}B is localised, i.e. within the tumour tissue. The course of treatment can be completed within 2-4 days. Slow neutrons

are non-ionizing. Also BNCT is capable of destroying diffuse tumours which are normally not clearly demarcated.

BNCT is not dependent upon oxygen levels in the tumour as many are hypoxic and can also be used where the cancer treatment is anatomically compromised. Deep-seated tumours can be treated at anything from 4-8 cm depth and beyond.

BNCT is less demanding for the patient than conventional radiotherapy as it can be given several times over a period of 2-4 days while in contrast conventional radiotherapy needs to be given up to 30 times over a period of six weeks.

In such a binary system, each component may be manipulated independently of the other. The interval between administration of the ^{10}B agent and neutron irradiation may be adjusted to an optimum time to provide the highest differential ^{10}B concentrations between normal tissues and the tumour tissue. Similarly, the neutron beam may be collimated so that the field of irradiation is limited to the tumour site and normal tissues having some residual ^{10}B concentration may be excluded from the treatment volumes.

However to obtain the potential benefits of NCT as an "in situ" cellular radiotherapy, there are a number of prerequisites. The neutrons must be of an energy range where only the NCT nuclides, for example the ^{10}B atoms, are able to undergo a fission reaction. The NCT agent must also be selectively localised in the target tissue. For example, 15-35 $\mu\text{g/g}$ of tumour tissue (equating to 10^9 boron atoms) is widely quoted to be the amount of ^{10}B necessary for effective BNCT using a neutron beam from a nuclear reactor of fluence 10^9 neutrons per second per cm^2 .

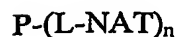
However, large concentrations of the boron-containing drugs need to be administered to achieve the 10^9 boron atoms per tumour cell. To illustrate this point, Table 1 below summarises three BNCT compounds that have been the subject of much preclinical and clinical research. BPA and BSH are in clinical use and the third is an experimental compound in preclinical development.

Table 1.

Compound	Absolute tumour concentration ($\mu\text{g/g}$)	Tumour:blood Ratio	Tumour:CNS ratio	Dose
BPA (in clinical trials)	16.5	2.2:1.0	1.7:1.0	900-1200 mg/kg (30 mg/ml)
BSH (in clinical trials)	6.9	0.7:1.0	3.0:1.0	-
CuTCPH (preclinical development)	114.0	570:1.0	57:1.0	200 mg/kg (6 mg/ml)

However, the above compounds need to be administered in extremely high concentrations for example up to 1200 mg per kg for BPA in a 30 mg/ml intravenous solution. This is a major concern and a disadvantage for the ill patient as there is a need to administer several litres of drug within a few hours to achieve the meager tumour to blood differential of 3:1. As a result patients are exposed to cardiovascular shock. To achieve this tumour to blood differential infusion protocols of 1, 2, 4, 6 and 8 hours are necessary. Also BPA and BSH have no selectivity for tumours. CuTCPH has higher tumour to blood ratios but suffers from the need to administer a 200 mg/kg dose which again is extremely high and would require about 15 g of compound in 100-1000 mL of liquid. New compounds are clearly required that overcome these shortcomings. However, after over 50 years of research and development effort, only two sub-optimal compounds (BPA and BSH) have entered clinical trials.

Accordingly, the present invention provides a conjugate having the general formula



wherein

P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a molecular weight of 5-6,000 kDa;

NAT represents a nuclide activation therapy agent;

5 L represents a linker moiety capable of linking the polymer to the neutron capture therapy agent; and

n represents an integer from 1-1,000.

10 The present invention thus provides a conjugate with improved tumour targeting compared to previously available compounds.

The present invention also provides a pharmaceutical composition containing the conjugate as defined above.

15 The present invention further provides the use of the conjugate as defined above for the preparation of a medicament for the treatment of cancer, as well as the use of the conjugate as defined above for the treatment of cancer. Preferably the invention relates to the treatment of solid tumours, such as brain, breast, head and neck, prostate, lung, bone, pancreas and liver, and colon cancers. In addition, the present invention provides a
20 method for treating cancer comprising administering a conjugate as defined above to a patient followed by activating the NAT agent at the site of the tumour.

The compounds of the present invention are large molecules dissolved in an aqueous medium suitable for parenteral administration. The polymer-NAT conjugates have
25 molecular weights in excess of 5 kDa compared with conventional drugs of 200-500 Da. These compounds on their own do not have any anti-tumour activity but in the presence of an activation source, e.g. neutrons, the NAT reaction takes place to generate the toxic high LET particles (α particles and lithium ions).

30 The polymer-NAT conjugates contain three components, namely a polymer, an NAT agent and a linker.

The polymer is a biocompatible (e.g. non-immunogenic and non-thrombotic, i.e. does not interfere with platelets and clotting factors), typically water soluble natural or synthetic polymer. The conjugate has a water solubility greater than 0.1 mg/ml, more preferably 1-100 mg/ml, most preferably 10-50 mg/ml. The polymer is pharmaceutically inert.

The polymers are required to have a blood-plasma half-life of 0.1 to 24 hours, preferably 0.2 to 12 hours, particularly preferably 1-6 hours. Sufficient half-life is required for the molecules to enter a cell via pinocytosis. Pinocytosis is a slow process whereby extracellular fluid (containing the drug molecule) is taken into the cytoplasm.

The molecular weight and shape of the molecule (e.g. string or a globule) will determine the filtration rate out of the kidney. The molecular weight of the polymer is 5-6,000 kDa, preferably 5-100, more preferably 10-70, more preferably 15-45, most preferably 20-40 kDa.

The polymer is water-soluble, preferably with a water solubility greater than 0.1 mg/ml, more preferably 50 mg/ml, most preferably greater than 100 mg/ml. These solubilities are necessary so when the NAT active agent (these normally have a poor water solubility) is conjugated the overall effect on the solubility of the compound is normally negligible. Water solubility also allows a significant decrease in the volume of drug solution which needs to be administered to the patient. It also negates the requirement of potentially toxic co-solvents in drug formulations.

However, low water solubility polymers are also useful (solubility below 0.1 mg/ml) but require the use of pharmaceutical excipients, such as oils, surfactants and/or emulsifiers, to generate a sufficiently concentrated liquid injectable system.

The polymer may be modified by introducing amino acids or artificial sugars which do not undergo enzymatic degradation, in order to enhance the stability of the polymer and increase the half-life.

The amount of the two monomers in the copolymer may be varied. The ratio of hydroxypropylmethacrylamide to methacrylate is preferably from 100:1 to 1:1, most preferably 20:1 to 1:1.

5

The NAT active nuclide must be capable of undergoing nuclear fission to produce particles which are sufficiently energetic to destroy tumour cells but which confine the cell damage to the diameter of a tumour cell, i.e. 10 μm . Such nuclides are known in the art and are exemplified by ^6Li , ^{10}B , ^{22}Na , ^{58}Co , ^{113}Cd , ^{126}I , ^{135}Xe , $^{148\text{m}}\text{Pm}$, ^{149}Sm , ^{151}Eu , ^{155}Gd , ^{157}Gd , ^{164}Dy , ^{184}Os , ^{199}Hg , ^{230}Pa , ^{235}U and ^{241}Pu . More preferably the nuclide is ^6Li , ^{10}B , ^{22}Na , ^{58}Co , ^{113}Cd , ^{126}I , ^{135}Xe , $^{148\text{m}}\text{Pm}$, ^{149}Sm , ^{151}Eu , ^{155}Gd , ^{157}Gd , ^{164}Dy or ^{184}Os . Typically, the particular element is enriched with the NAT active nuclide. The NAT active nuclides are attached to the polymer via compounds which carry the NAT active nuclide. These compounds are known in the art and are exemplified below:

15

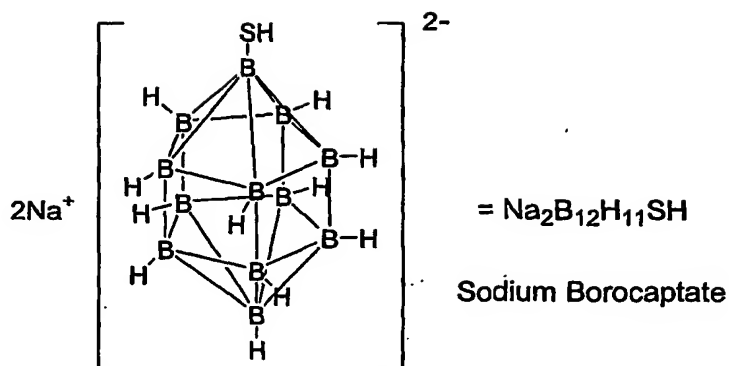
Boronated amino acids and peptides, e.g. boronophenylalanine (BPA), see Soloway Chem. Rev. (1998) Vol 98, No. 4, pages 1531-1534 and Snyder, H R et al J. Am. Chem. Soc. (1958) 80, 835;

20

Modified carborane cages, such as $[\text{B}_{10}\text{H}_{10}]^{2-}$ (decahydrodecaborate) and $[\text{B}_{12}\text{H}_{12}]^{2-}$ (dodecahydrododecaborate), e.g. $\text{C}_2\text{B}_{12}\text{H}_{12}$, see Hawthorn, M F et al J. Am. Chem. Soc. (1959) 81, 5519 and Grimes, R. N. in "Carboranes" Academic Press NY (1970);

Mercaptoborates, such as mercaptoundecahydrododecaborate ($\text{B}_{12}\text{H}_{11}\text{SH}^{2-}$) (BSH), the structure of the disodium salt is shown below, which may also be in the form of a dimer (BSSB), see Soloway, A. H. et al J. Med. Chem. (1967) 10, 714;

25

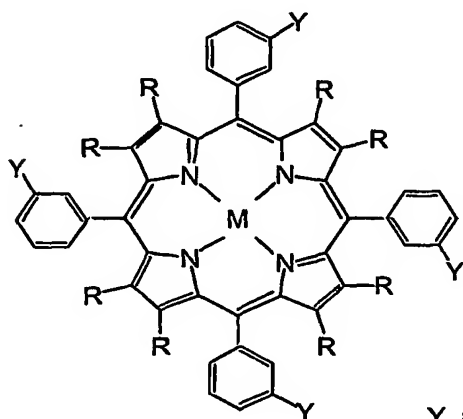


Porphyrins and phthalocyanines, e.g. BOPP and LTCPs (NiTCP, CuTCP, NiTCPH and CuTCPH) in which the porphyrins carry four carborane cages, see Ozawa, T. Pro. Am. Ass. for Cancer March 1998, 39, p586 and Miura, M., Radiation Research (2001) 155, 603-610 (see structures below);

Boron-containing nucleic acid precursors, such as boronated and carborane-containing pyrimidines and purines, e.g. 5-(dihydroxyboryl) uracil, 5-carboranyluracil, see Liao, T.K. J. Am. Chem. Soc. (1964) 86, 1869, Schinazi, R. F. J. Org. Chem. Soc. (1985) 50, 841, and Nemoto, H. J. Chem. Soc. Chem. Commun. (1994) 577; and

- 10 Foliates, growth factors, hormones, radiation sensitisers, phosphates, phosphonates and phosphoramidates, cyclic thiourea derivatives, amines, promazines, hydantoins, barbiturates, see Soloway Chem. Rev. (1998) Vol 98, No.4, pages 1545-1550.

Structure of various lipophilic carboranyltetraphenylporphyrins.

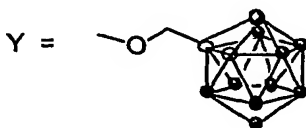


NiTCP, $\text{R} = \text{CH}_2\text{CO}_2\text{CH}_3$, $\text{M} = \text{Ni}$

CuTCP, $\text{R} = \text{CH}_2\text{CO}_2\text{CH}_3$, $\text{M} = \text{Cu}$

NiTCPH, $\text{R} = \text{H}$, $\text{M} = \text{Ni}$

CuTCPH, $\text{R} = \text{H}$, $\text{M} = \text{Cu}$



R may also represent halogen, preferably Br or Cl, or a nitro group (NO).

Other nuclides may be incorporated in the same manner. For example, a combined BNCT and GdNCT compound where the Gd nuclide and a carborane cage are on the same molecule is known in the art, see Soloway (1998) Chem. Rev. Vol 98, No. 4, page 1519.

The NAT agent should preferably make up 1-30%, preferably 5-10% of the overall mass of the polymer-NAT conjugate.

The NAT agent is activated using known techniques, such as irradiation with electromagnetic radiation (e.g. X-rays, light, microwaves, gamma rays) or by neutrons or by ultrasound, protons, carbon ions, pion therapy, electron beam therapy, antiproton therapy, photon therapy, photodynamic therapy. In the case of neutrons, the technique is termed neutron capture therapy and the agent is termed a neutron capture therapy (NCT) agent.

The linker may be any group which links the polymer and the NCT agent and which does not effect the in vivo solubility or toxicity properties of the polymer-NAT conjugate.

Such linkers include linear or branched C₁₋₁₅ alkyl which may be saturated or unsaturated, optionally substituted by carbonyl, amide, hydroxyl or halogen, such as methyl, ethyl, propyl, n-butyl, i-butyl, t-butyl, 1-methylbutyl and methylpentyl; and peptides, preferably 1-10 amino acids in length in which the amino acids may be further substituted with amino, thio, carboxyl, carboxamide or imidazole groups. Preferred peptides are Gly-Gly, Gly-Phe-Gly, Gly-Phe-Phe, Gly-Leu-Gly, Gly-Val-Ala, Gly-Phe-Ala, Gly-Leu-Phe, Gly-Leu-Ala, Ala-Val-Ala, Gly-Phe-Leu-Gly, Gly-Phe-Phe-Leu, Gly-Leu-Leu-Gly, Gly-Phe-Tyr-Ala, Gly-Phe-Gly-Phe, Ala-Gly-Val-Phe, Gly-Phe-Phe-Gly, Gly-Phe-Leu-Gly-Phe and Gly-Gly-Phe-Leu-Gly-Phe. Particularly preferred peptides are Gly-Gly and Gly-Phe-Leu-Gly.

Some of the peptide linkers may be degraded by lysosomal enzymes so that the NAT compound is released within the tumour cell.

The linker is attached to the polymer and the NAT agent by conventional synthetic methods well known to the skilled person. The following bonds provide a suitable means for attaching the NAT agents to the polymer: an amide bond, an ester bond, a hydrazide bond, a urethane (carbamate) bond, a carbonate bond, an imine (Schiff base) bond, a thioether bond, an azo bond or a carbon-carbon bond. Alternatively, the NAT agent may be attached directly to the polymer itself, i.e. the linker is a covalent bond.

Amide bonds may be made using an amino group ($-NH_2$) and a carboxylic acid group ($COOH$). The latter should be transformed to a more reactive intermediate such as an acid chloride ($COCl$) or using coupling agents such as carbonyl diimidazole (CDI) or dicyclohexylcarbodiimide (DCC). Other substrates for the amino group are acid anhydrides and esters. Ester bonds may be made from hydroxyl groups (OH) and the activated carboxylic acids mentioned above and acid anhydrides. Hydrazide bonds may be made from acyl halides (such as the acid chloride above) and a hydrazine (NH_2NHR). Carbamate bonds may be made from phosgene ($ClCOCl$) or, preferably, trichloromethyl chloroformate (CCl_3OCOC), which may be treated with an alcohol and an amine. Carbonate bonds may also be made from phosgene or trichloromethyl chloroformate, which may be treated with two alcohol groups. Imines (or Schiff bases) may be made by condensation between an aldehyde ($RCHO$) or ketone ($RCOR$) and an amine. Aldehydes are easily made by oxidation of primary alcohols and ketones may be made by oxidation of secondary alcohols. Thioethers may be made by first converting a group such as an alcohol, into a good leaving group, such as a tosylate, mesylate, triflate or halide, and treating it with a mercaptide (RS^-), which substitutes the leaving group with RS . See, for example, Schacht E (1987) in: Illum L, Davis SS (eds) "Polymers in drug delivery", Wright, Bristol, p131.

Typically, the linker is attached to the polymer at the methacrylate monomers, preferably via an amide bond. Methacrylate monomers which do not have pendant NAT agents (or

any other pharmaceutically active agent) may still have a linker which may be capped using a capping compound, for example 2-amino-1-propanol.

For effective therapy planning and BNCT dose calculations, it is important to know the location and concentration of the drug in the tumour. Therefore, in the clinical setting as part of the treatment planning it is important to know the site and the amount of the NAT agent in the tumour mass in order to calculate the clinical neutron dose. For example, a macromolecule may carry a metal which can be imaged using diagnostic techniques, such as PET, SPECT, MRI. The lipophilic carboranyltetraphenylporphyrins (LCTP), for example, are able to carry a metal ion. Such a metal may be selected from vanadium (V), manganese (Mn), iron (Fe), ruthenium (Ru), technetium (Tc), chromium (Cr), platinum (Pt), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), germanium (Ge), indium (In), tin (Sn), yttrium (Y), gold (Au), barium (Ba), tungsten (W), gadolinium (Gd) and gallium (Ga). The most preferred metals are Cu and Ni.

Positron Emission Tomography (PET) may also be used to provide an indication of the position and concentration of the polymer-NAT conjugate in the tumour. In PET, radio nuclides such as ^{18}F (half-life of about 2 hours) or ^{11}C (half-life of 20 minutes) are preferred. A single fluorine atom may be incorporated into the polymer backbone or ideally in an amino acid, preferably Phe, in the linker chain. This provides a very powerful tool for diagnosis and treatment planning enabling customised individual polymer-NAT conjugate drug dosing. The presence of a fluorine atom provides a means of substituting the F for the radionuclide ^{18}F at the time of diagnosis using well known PET imaging chemistry.

Some preferred examples of polymer-NAT conjugates of the present invention will now be described.

Preferably, the polymer is a copolymer of N-(2-hydroxypropyl)methacrylamide (HPMA) and methacrylic acid; the linker is a peptide, such as Gly-Phe-Leu-Gly; and the NAT agent is selected from o-carboranylalanine $\text{B}_{10}\text{C}_2\text{H}_2\text{-CH}_2\text{CHCO}_2\text{NH}_2$, carborane butamine

$B_{10}C_2H_2-(CH_2)_3CHCO_2NH_2$, BPA (p-boronophenylalanine), $B_{12}H_{11}SH$ (BSH) (mercaptoundecahydrododecacarborate), boronated porphyrins, BSH-glutathione disulfide, and water soluble tetracarbonylphenylporphyrin eg. NiTCP.

5 Particularly preferred conjugates are:

HPMA-co-MA-Gly-Phe-Leu-Gly-BSH

HPMA-co-MA-Gly-BPA-Leu-Gly-BPA

HPMA-co-MA-Gly-BPA-Leu-Gly-Gly-BPA

10 HPMA-co-MA-Gly-Phe-Leu-Gly-Carborane butamine ($B_{10}C_2H_{11}(CH_2)_3CHCO_2NH_2$)

HPMA-co-MA-Gly-BPA-Leu-Gly-Carborane butamine ($B_{10}C_2H_{11}-(CH_2)_3CHCO_2NH_2$)

HPMA-co-MA-Gly-Phe-Leu-Gly-CuTCPH

HPMA-co-MA-Gly-Phe-Leu-Gly-CuTCPHBr

15 The polymer-NAT conjugates of the present invention are selectively targeted to tumours and exploit a number of tumour properties. For example, the tumour has a negative osmotic pressure since there is no lymphatic drainage, leading to trapping of the polymer-NAT conjugate (the so-called Enhanced Permeability Retention (EPR) effect). The vast majority of small compounds thus enter into the tumour via diffusion and tend to remain

20 in the tumour bed and move between the cells through gap junctions. The compounds normally enter the cells by endocytosis into the cytoplasm and end up in the lysosomal compartment. The polymer-NAT conjugates are targeted to the cell membrane, the cytoplasmic organelle, e.g. mitochondria, endothelial reticula or Golgi apparatus, and/or the nucleus. The polymer-NAT conjugate being nearer to the nucleus and cellular

25 organelles increases the probability of irreversible DNA or cellular damage from the ionising particles (e.g. α and Li ion) resulting from the fission of the NAT agent.

The polymer component of the polymer-NAT conjugate may also be modified to target particular areas of the body. For example, the asialoglycoprotein receptor on hepatocytes

30 can recognise both galactose and N-acetylgalactosamine. Accordingly, incorporating

these components into the polymer allows polymer-NAT conjugate of the present invention to be directed to the liver.

Galactose may be incorporated in to the monomer of the HPMA with a protected hydroxyl group. For example, 1,2,3,4-di-O-isopropylidene-6-O-methacryloyl- α -D-galactopyranose is synthesised and then copolymerised with HPMA followed by removing the protecting group, isopropylidene, with formic acid. See, Chytry, V. et al New Polymer Material (1987) 1, 21. N-acylated galactosamine is attached using the reactive HPMA copolymer precursor having side chains terminated with p-nitropyridine esters. These are aminolyzed with galactosamine in DMSO at room temperature and pressure.

In addition, the non-specific uptake of the polymer-NAT conjugate may be enhanced by incorporating a positive charge (e.g. using methacryloxylethyltrimethylammonium chloride) or hydrophobic comonomers (e.g. using N-[2-(4-hydroxyphenylethyl)] acrylamide or N-methacryloyltyrosinamide) into the polymer.

NAT requires that a polymer-NAT conjugate is administered at a dose of 0.1-100 mg/kg body weight, preferably 0.1-50 mg/kg, particularly preferably 1-30 mg/kg. The dose should deliver at least 10 μ g/g of active nuclide, e.g. 10 B atoms, per gram of wet tumour tissue, preferably at least 25 μ g/g, more preferably at least 80 μ g/g, more preferably at least 160 μ g/g and most preferably over 200 μ g/g.

Incidentally, the polymer-NAT conjugate of the present invention provides a significant reduction in systemic toxicity compared to known NAT agents and hence a higher dose may be given leading to a greater concentration of the NAT agent inside the tumour cell. Toxicity is reduced since the NAT agent within the polymer-NAT conjugate is not available for interaction with the biological environment. In addition, since the polymer-NAT conjugate is retained by the tumour for long periods of time, i.e. several hours to days, there is no need for multiple dosing thereby widening the clinical treatment window.

In addition to the NAT agent, other drugs, or their prospective prodrugs, may be incorporated into the polymer-NAT agent of the present invention thereby providing increased anti-cancer activity. That is, a polymer-NAT conjugate having the general formula $P-(L-NAT)_n(L\text{-chemotherapeutic agent})_m$ wherein P, L, NAT and n have the same meaning as defined above, L may be the same or different and m is an integer from 1-1000, preferably 1-500, more preferably 1-100, most preferably 1-20. Such chemotherapeutic agents may include, for example, any one of the following: 5-(aziridin-1-yl)-4-dihydroxylamino-2-nitrobenzamide, phenylenediamine mustard, benzoic acid mustards, ganciclovir triphosphate, adenine arabinonucleoside triphosphate (araATP), hydrogen peroxide, cyanide, superoxide, methotrexate, mitomycin alcohol, etoposide, palytoxin, melphalan, 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, actinomycin D, mitomycin C, taxanes, such as taxol and taxotere, topoisomerase inhibitors, such as camptothecin and topotecan cyclophosphamide, carmusline, 5-fluorouracil, cytarabine, mercaptopurine, anthracyclines, daunorubicin, doxorubicin, epirubicin, vinca alkaloids, vinblastin, vincristine, dactinomycin, mitomycin C, lasparaginase, G-CSF, cisplatin and carboplatin.

A preferred conjugate of this type is HPMa-co-MA-[Gly-Phe-Leu-Gly-BSH](Gly-Phe-Leu-Gly-Y)], where Y is the anticancer agent, for example:

HPMA-co-MA [(Gly-Phe-Leu-Gly-BSH)(Gly-Phe-Leu-Gly Doxorubicin)]

HPMA-co-MA [(Gly-Phe-Leu-Gly-BSH)(Gly-Phe-Leu-Gly Ellipticin)]

HPMA-co-MA [Gly-Phe-Leu-Gly-BSH](Gly-Phe-Leu-Gly Cisplatin)]

A feature of the polymer-NAT conjugates of the present invention is that they build up in a tumour without the need for a targeting moiety attached to the polymer. However, the polymer-NAT conjugate, with or without an active anti-cancer chemotherapeutic agent, may contain a targeting moiety attached to the polymer. These targeting moieties are selected to increase the concentration of the compound at the desired target tissue. Such moieties significantly change the biodistribution of the conjugate by providing a means of

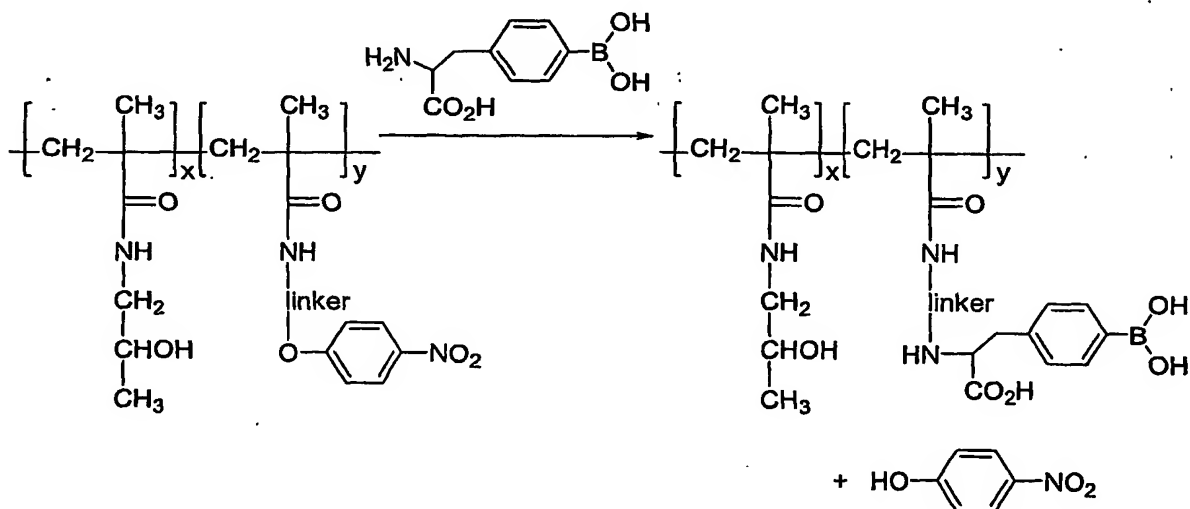
selectivity. Such targeting moieties include (N-acylated) galactosamine, (6-O-bound) galactose, (N-acylated) fucosylamine, melanocyte stimulating hormone, and secretin.

The present invention will now be illustrated by a number of examples which describe a new class of polymer-NAT conjugates which overcome the shortcomings of current BNCT agents.

Examples

- 10 Polymers were obtained from Polymer Laboratories Ltd. The certificates of analysis were obtained from Butterworth.

In examples 1 and 2 below, reaction of 4-boronophenylalanine (BPA) with the p-nitrophenyl ester group of the polymer leads to the formation of a boron-containing polymer with the displacement of p-nitrophenol as depicted by the reaction scheme below.



Commercially available poly(HPMA-co-MA-GG-ONp) from Polymer Laboratories Ltd has an average molecular weight of 28,100 with a broad molecular weight distribution, weight average molecular weight/number average molecular weight, $M_w/M_n = 1.31$, leading to average values of $x = 153$, $y = 17$ (MW = 27,624). Poly(HPMA-co-MA-

GFLG-ONp) has an average molecular weight of 47,200 ($M_w/M_n = 1.53$). From the average molecular weight, average values of x and y may be calculated as $x = 225$, $y = 25$ ($MW = 47,131$). These values have been used to calculate how much of the boron substituted phenylalanine should be added.

5

Example 1

Gram-scale preparation of poly(HPMA-co-MA-GG-F[4-B(OH)₂])

Powdered poly(HPMA-co-MA-GG-ONp) (1.1 g, 40.0 μmol) and 4-boronophenylalanine
10. (¹⁰B enriched) (0.15 g, 740 μmol) were placed in a dried flask which was sealed with a
septum and flushed with argon. Anhydrous DMSO (11 mL) was added and the mixture
was stirred until all the material had dissolved to produce a cloudy solution.
Triethylamine (4 drops) as a catalyst was added causing the solution to become yellow.
The solution was stirred at 20-22°C (oil bath temperature) overnight under argon. The
15 solution was diluted with diethyl ether (200 mL) and the solvent was decanted off leaving
a solid sticky precipitate. The solid sticky precipitate was triturated with further portions
of diethyl ether until it was no longer sticky. Residual solvent was evaporated in vacuo
(ca 0.4 mmHg) at 36°C for 3h. The product poly(HPMA-co-MA-GG-F[4-B(OH)₂]) was
obtained as a beige/yellowish solid (1.20g, 104%), ¹H NMR analysis revealed the
20 presence of some residual diethyl ether and DMSO.

Boron analysis was done by Butterworth Laboratories Ltd. It was found that boron
content of the poly(HPMA-co-MA-GG-F[4-B(OH)₂]) was 0.47% (the expected was
0.59%). The low values could be due to incomplete reaction of the polymer with 4-
25 boronophenylalanine and the presence of residual solvent.

Example 2

Gram-scale preparation of poly(HPMA-co-MA-GFLG-F[4-B(OH)₂])

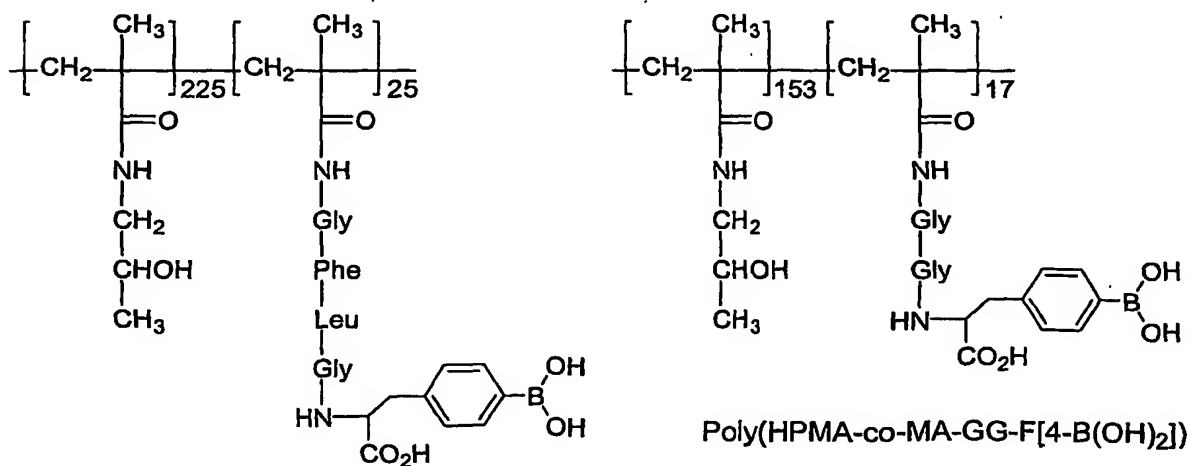
30 Powdered poly(HPMA-co-MA-GFLG-ONp) (1.1g, 23.3 μmol) and 4-
boronophenylalanine (¹⁰B enriched) (0.13 g, 625 μmol) were placed in a dried flask

which was sealed with a septum and flushed with argon. Anhydrous DMSO (11 mL) was added and the mixture was stirred until all the material had dissolved to produce a cloudy solution. Triethylamine (4 drops) as a catalyst was added causing the solution to become yellow. The solution was stirred at 20-22°C (oil bath temperature) overnight under argon.

- 5 The solution was diluted with diethyl ether (200 mL) and the solvent was decanted off leaving a sticky precipitate. The sticky precipitate was triturated with further portions of diethyl ether until it was no longer sticky. Residual solvent was evaporated in vacuo (ca 0.4 mmHg) at 36°C for 3h. The final product poly(HPMA-co-MA-GFLG-F[4-B(OH)₂]) was obtained as a beige/yellowish solid (1.23 g, 102%), ¹H NMR analysis revealed the
10 presence of some residual diethyl ether and DMSO.

Boron analysis was done by Butterworth Laboratories Ltd. It found that the polymer contains 0.40% boron (the expected was 0.48%). These slightly low values could be due to incomplete reaction of the polymer with 4-boronophenylalanine and the presence of
15 residual solvent.

The chemical structures of the polymer-NAT conjugates prepared in examples 1 and 2 are:



Poly(HPMA-co-MA-GFLG-F[4-B(OH)₂])

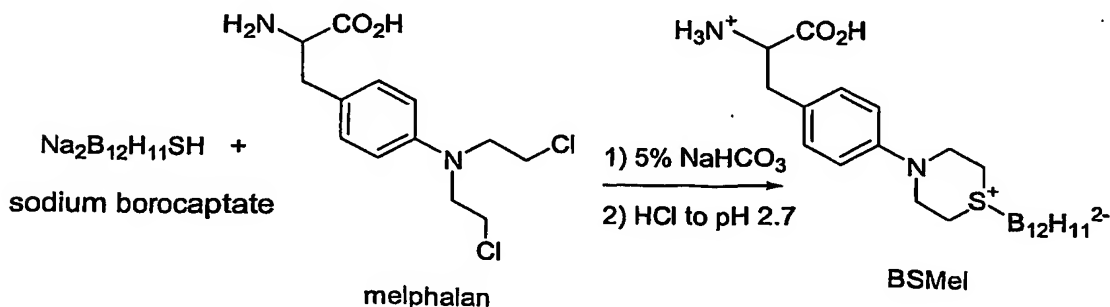
The polymer-NAT conjugates of examples 1 and 2 only differ in their peptide linkers. The polymer-NAT conjugate of example 1 has the peptide linker Gly-Phe-Leu-Gly which is enzymatically degraded in the lysosomal compartment of a cell to release the BPA whereas the polymer-NAT conjugate of example 2 has the peptide linker "Gly-Gly" which is not degraded leaving the entire molecule intact. The biodegradable polymer is able to release the boron-carrying molecule into the cytoplasm and has the opportunity to diffuse into the heart of cellular organelles and most importantly into the nucleus. In BNAT, the closer the boron carrier molecule is to the DNA the more effective the cell kill. However, with the non-degradeable polymer-NAT conjugate, the molecule remains intact and is unable to leave the cell once it has been internalised. This allows a build up of a very high concentration of the boronated polymer in a cell via multiple doses which otherwise may not be possible due to systemic toxicity of the boronated polymer.

Example 3

Preparation of poly(HPMA-co-MA-GG-F BSMel) (Applicant's code PP403)

The poly (HPMA-co-MA-GG-F BSMel) was synthesised using commercially available sodium borocaptate. The sodium borocaptate was first converted to a phenylalanine derivative, sodium boronocaptate melphalan (BSMel) in "Step 1" using the procedure described in US 6,017,902. This step was followed by "Step 2" in which BSMel was reacted with poly(HPMA-co-MA-GG-ONp) to yield the poly (HPMA-co-MA-GG-F BSMel).

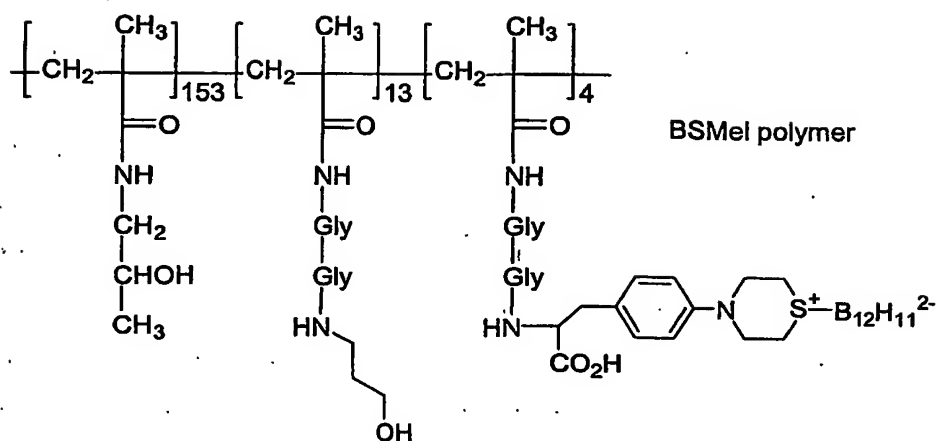
Step 1 Preparation of BSMel



Sodium borocaptate (0.95 g, 4.32 mmol) was dissolved in a 5% sodium hydrogen carbonate solution (30 mL). Melphalan, a yellowish solid, (0.22 g, 0.72 mmol) was added and the suspension was stirred at room temperature over a 48 h. The yellowish solid melphalan dissolved completely and a fine white precipitate was formed. The mixture
5 was chilled (ca 4°C) for 3 h and then filtered through a nylon membrane. The resultant solid was dissolved in water (30 mL) with heating (ca 100°C bath temperature). The resultant solution was allowed to cool to room temperature and was filtered. The pH of the solution was adjusted to 2.7 by careful dropwise addition of conc. hydrochloric acid causing the precipitation of a fine solid. After chilling overnight (ca 4°C), the product
10 was collected by filtration and washed with cold water. Residual solvent was evaporated in vacuo (ca 0.4 mmHg) at ca 30°C for 5 h. The product was obtained as a light tan solid (0.19 g, 65%).

Step 2 Preparation of BSMel Polymer

15 Powdered poly(HPMA-co-MA-GG-ONp) (1.1 g, 40.0 µmol) was placed in a dried flask which was sealed with a septum and flushed with argon. Anhydrous DMSO (11 mL) was added and the mixture was stirred until all the material had dissolved. BSMel (0.069 g, 169 µmol) from Step 1 was added. When all the solid had dissolved, triethylamine (2
20 drops) was added causing the solution to become yellow. The solution was stirred at 20-22°C (oil bath temperature) overnight under argon. 3-Amino-1-propanol (41 µl, 536 µmol) was added and the solution was stirred for a further 3 h. The solution was diluted with diethyl ether (200 mL) and the solvent was decanted off leaving a sticky precipitate. The yellow solid was triturated with further portions of diethyl ether until it was no
25 longer sticky. Residual solvent was evaporated in vacuo. Yield 1.10 g.

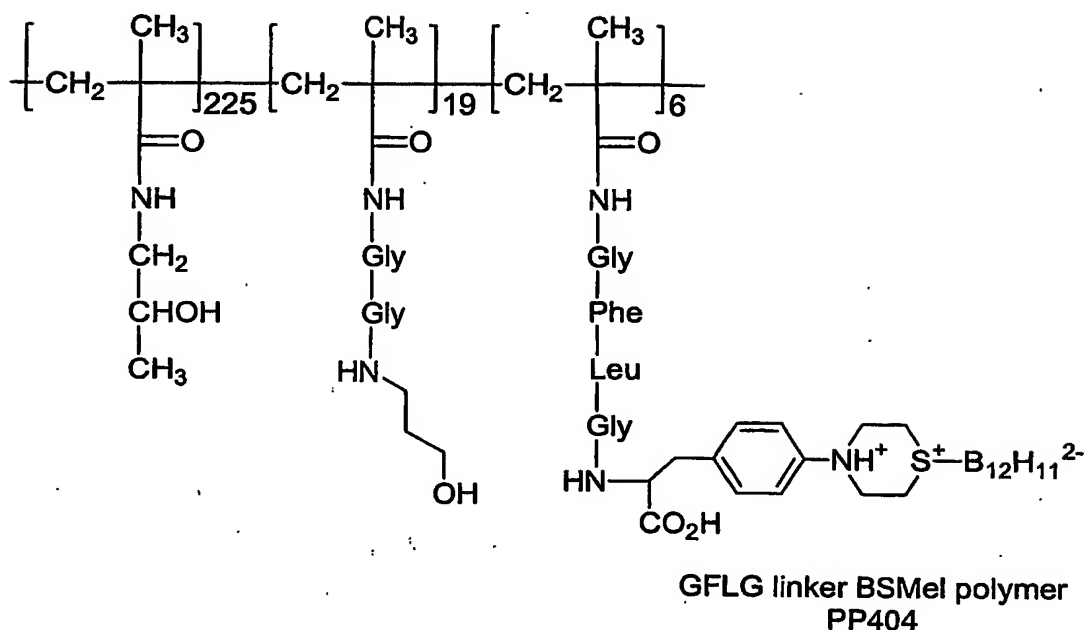


This non-biodegradable boron-carrying polymer is able to deliver 12 boron atoms per boron carrier and there are between 3-4 BSMel molecules per polymer molecule. Therefore, the BSMel polymer is able to deliver in excess of 30-40 times more boron per polymer molecule than those in Examples 1 and 2. This provides a very powerful way of delivering a high concentration of boron with a comparatively low concentration of polymer.

Example 4

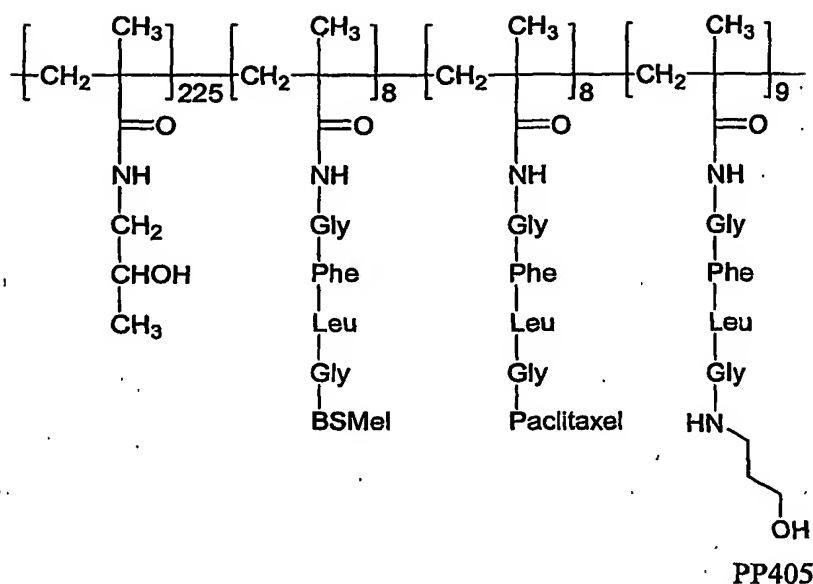
10 Preparation of poly(HPMA-co-MA-GFLG-BSMel) (PP404)

Powdered poly(HPMA-co-MA-GFLG-ONp) (1.1 g, 23.3 μmol) was placed in a dried flask which was sealed with a septum and flushed out with argon. Anhydrous DMSO (11 mL) was added and the mixture was stirred until all the material had dissolved. BSMel (0.062 g, 152 μmol) was added. When all the solid had dissolved, triethylamine (2 drops) was added causing the solution to become yellow. The solution was stirred at 20-22°C (oil bath temperature) overnight under argon. 3-Amino-1-propanol (35 μL , 456 μmol) was added and the solution was stirred for a further 24 h. The solution was diluted with diethyl ether (220 mL) and the solvent was decanted off the sticky precipitate. The off-white solid was triturated with further portions of diethyl ether until it was no longer sticky. Residual solvent was evaporated in vacuo. Yield 0.95 g.

**Example 5**

Preparation of poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMel)-Gly-Phe-Leu-Gly-
5 Paclitaxel (PP405)

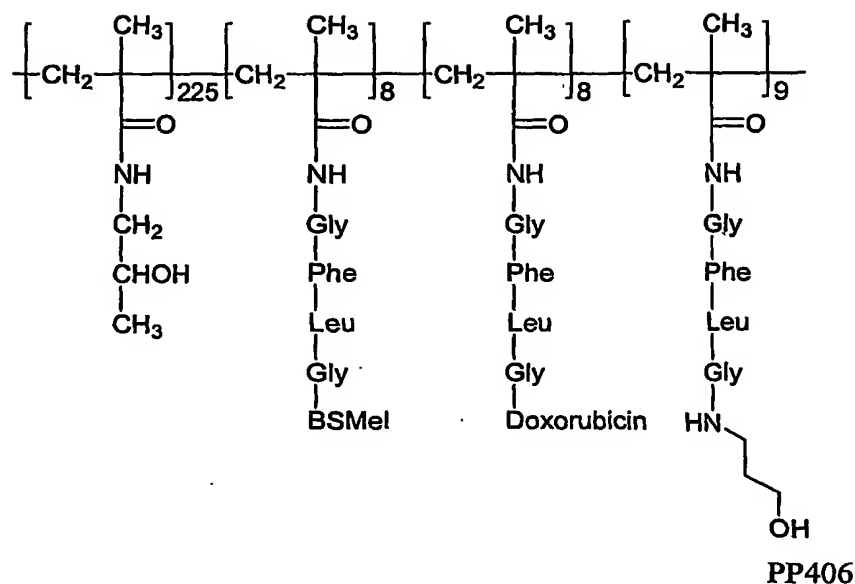
Powdered poly(HPMA-co-MA-GFLG-ONp) (2.15 g, 45.6 μmol) was placed in a dried flask which was sealed with a septum and flushed out with argon. Anhydrous DMSO (22 mL) was added and the mixture was stirred until all the material had dissolved. BSMel
10 (0.146 g, 366 μmol) was added. When all the solid had dissolved, triethylamine (52 μL , 366 μmol) was added causing the solution to become yellow. The solution was stirred at 20-22°C (oil bath temperature) under argon for 5 h. Paclitaxel (0.313 g, 366 μmol) and 4-dimethylaminopyridine catalyst (0.015 g, 123 μmol) were added. After stirring overnight at 20-22°C, 3-amino-1-propanol (35 μL , 456 μmol) was added and the solution was
15 stirred for a further 4h. The solution was slowly poured into stirred diethyl ether (500ml) and the solvent was decanted off the sticky precipitate. The yellowish solid was triturated with further portions of diethyl ether until it was no longer sticky. Residual solvent was evaporated in vacuo. Yield 2.44 g (98%).



Example 6

- 5 Preparation of poly(HPMA-co-MA-Gly-Phe-Leu-Gly-BSMel) Gly-Phe-Leu-Gly-Doxorubicin (PP406)

Powdered poly(HPMA-co-MA-GFLG-ONp) (2.15 g, 45.6 μ mol) was placed in a dried flask which was sealed with a septum and flushed out with argon. Anhydrous DMSO (22
 10 mL) was added and the mixture was stirred until all the material had dissolved. BSMel (0.146 g, 366 μ mol) was added. When all the solid had dissolved, triethylamine (52 μ L, 366 μ mol) was added. The solution was stirred at 20-22°C (oil bath temperature) under argon for 5h. Doxorubicin hydrochloride (0.212 g, 366 μ mol) and triethylamine (52 μ L, 366 μ mol) were added. After stirring overnight at 20-22°C, 3-amino-1-propanol (35 μ L,
 15 456 μ mol) was added and the solution was stirred for a further 4 h. The solution was slowly poured into stirred diethyl ether (500 mL) and the solvent was decanted off the sticky precipitate. The red solid was triturated with further portions of diethyl ether until it was no longer sticky. Residual solvent was evaporated in vacuo. Yield 2.55g (105%).



Biodistribution Studies

- 5 Female BALB/c mice (20-25 g) bearing the EMT-6 carcinoma were used. The mice were housed four to a cage in temperature-controlled rooms and had free access to food and water. The mice were maintained in a controlled light/dark cycle, with lights on between 0700 and 1900h. In all studies, anaesthesia was maintained with ketamine (120 mg/kg) and xylazine (20 mg/kg). Animals were monitored on a daily basis for general health. Animals
- 10 were euthanised, as required, under anaesthesia.

The two boronated polymers synthesized in Examples 3 and 4, namely PP403 and PP404, were injected into EMT-6 tumour-bearing mice via the tail vein. The compounds were injected in a saline solution containing 50 mg/ml (0.5 mg boron/ml). The administered

15 volume was 0.01 mL/gbw by single bolus injection delivering a dose of approximately 5 mg boron/kg.

Concentrations of boron in tissue samples were determined using direct current plasma atomic emission spectroscopy (DCP-AES), see Coderre, J.A., Button, T.M., Micca, P.L.

20 Fisher, C., Nawrocky, M.M., and Liu, H.B. *Int. J. Radiat. Oncol. Biol. Phys.* (1994) 30, 643-652.

Visual observation of animal appearance, levels of activity and general behaviour indicated no evidence of toxicity (see Miura, M., Micca, P.L., Fisher, C.D., Gordon, C.R., Heinrichs, J.C. et al. Brit. J. Radiol. (1998) 847, 773-781). Tumour, blood, brain, and liver were sampled at 6, 24, 48, and 72 hours after injection for boron analyses. Four mice were used per time sampling point (16 mice in total). The boron biodistribution data are detailed in Table 2 below.

The tumour boron concentration from PP403 was about three times greater than that from PP404 which is likely to be due to the non-degradable linker in PP403. The liver:tumor boron ratios were between 2:1 and 3:1 at all time points for PP403 and were between 4.5:1 and 6:1 for PP404. The tumor:blood boron ratios using PP403 were less than 1:1 until the 72 h time point, and were less than 1:1 until the 48 h time point for PP404. The absolute tumour boron concentrations are low, but the administered boron dose was also low. In comparison to biodistribution data using a similar boron dose from CuTCPh in CRM, the tumour uptake is about 2.3 times lower, but the liver uptake is 5 times lower.

Table 2.

Time after injection (h)	Blood ($\mu\text{g/g}$)	Tumour ($\mu\text{g/g}$)	Liver ($\mu\text{g/g}$)	Brain ($\mu\text{g/g}$)
PP403				
6	13.8 ± 0.5	3.5 ± 0.2	10.4 ± 0.5	0.3 ± 0.1
24	5.7 ± 0.1	3.1 ± 0.3	7.4 ± 0.4	0.2 ± 0.1
48	4.2 ± 0.5	2.9 ± 0.1	7.7 ± 0.4	0.1 ± 0
72	2.1 ± 0.2	2.3 ± 0.2	6.9 ± 0.2	0.1 ± 0.1
PP404				
6	3.0 ± 0.3	1.3 ± 0	7.6 ± 0.3	0.1 ± 0
24	1.2 ± 0.1	1.1 ± 0.1	5.0 ± 0.5	0.2 ± 0
48	0.7 ± 0.0	0.8 ± 0.1	4.9 ± 0.2	0.1 ± 0
72	0.5 ± 0	0.9 ± 0.1	5.4 ± 0.4	0.1 ± 0.1

Table 2 shows the conjugate is able to target tumours and release the NAT agent where there is a biodegradable linker.

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(71) Applicant (*for all designated States except US*): PSIMEI PHARMACEUTICALS PLC [GB/GB]; Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): PATEL, Bipin, C., M. [GB/GB]; Psimei Pharmaceuticals PLC, Compton House, Walnut Tree Close, Guildford, Surrey GU1 4TX (GB).

(74) Agent: ELKINGTON AND FIFE; Prospect House, 8 Pembroke Road, Sevenoaks, Kent TN13 1XR (GB).

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(54) Title: CONJUGATES OF N-HYDROXYPROPYLMETHACRYLAMIDE-METHACRYLATE COPOLYMER WITH NUCLIDE ACTIVATION AGENT AND/OR ANTI-CANCER COMPOUNDS

(57) **Abstract:** The present invention relates to new anti-cancer compounds and in particular to new compounds for use in nuclide activation therapy, such as neutron capture therapy. Specifically this invention provides a conjugate having the general formula P-(L-NAT)_n wherein P represents an N-hydroxypropylmethacrylamide-methacrylate copolymer having a molecular weight of 5-6,000 kDa; NAT represents a nuclide activation therapy agent; L represents a linker moiety capable of linking the polymer to the neutron capture therapy agent; and n represents an integer from 1-1,000.



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